Asia Dry Eye Society

CHINESE DRY EYE SOCIETY

长沙

第一届全国眼表与泪液疾病学术大会
暨第三届全国干眼学术会议

The 1st Chinese Ocular Surface & Tear Film Diseases Congress in conjunction
The 3rd Chinese Dry Eye Congress

聚焦临床 促进转化

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observed clearly with IVCM. Demodex infection in the MG orifice is directly correlated with MG dysfunction, which warrants greater attention.

A-004 Pharmacology of Fibrinogen-depleted Human Platelet Lysate as a Treatment for Dry Eye Syndrome

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【摘要】:

Purpose: Dry eye disease was re-defined in the June 2017 Tear Film Ocular Surface Society (TFOS) Dry Eye Workshop (DEWS II) as "a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles."

The ideal topical product would restore healthy conditions of the eye surface and facilitate tissue repair. Clinical use of autologous serum drops (ASD) for the treatment of ocular surface disorders dates back to the 1970's when it was first used to treat ocular alkali burns delivered to the ocular surface through mobile perfusion pump [1]. In 1984, use of autologous serum eye drops was first described in the literature in patients with dry eyes [2]; however, it was not until late 1990's that the use of ASD became more widespread owing much to research performed by Tsubota et al. [3,4]. Serum, rich in growth factors and nutritive components, and naturally depleted of fibrinogen, is part of normal wound healing and tissue regeneration. Serum-treated cells in injured tissues proliferate and migrate to re-establish normal epithelial tissues. Autologous serum has been used anecdotally to treat dry eye in Sjögren's syndrome and ocular graft-versus-host disease, but a consistent manufacturing process approved by regulatory authorities has not been established. Compared to autologous serum, fibrinogen-depleted human platelet lysate (FDhPL, UltraGRO™-Advanced and UltraGRO™-PURE, Helios, USA; Aurarix™,Cambium Medical Technologies, USA) is richer in EGF and PDGF-BB and other nutritive components that can improve the growth of corneal epithelial cells. This study evaluates the pharmacology of FD hPL with respect to migration and growth of human corneal epithelial cells.

Methods: Enzyme-linked immunosorbent assay (ELISA) evaluated EGF (DuoSet®ELISA DY236) and PDGF-BB (DuoSet®ELISA DY220) levels in FDhPL. Protein content was determined by BCA assay. FDhPL was diluted into Plasmalyte A (Baxter Industries,USA) at concentrations of 10% and 30% v/v and the in vitro growth-promoting activities of diluted FD

hPL mixed 1:1 with serum-free HCEC media were compared to 5% FBS/HCEC media on cultures of human corneal epithelial cells (HCEC, ATCC®CRL-11135™). A MTT assay evaluated cell viability and a migration assay investigated wound healing.

Results: FD hPL has 3.83 ng/ml EGF and 17.7 ng/ml PDGF-BB. Total protein content was 51.4 mg/ml. (Table 1.) 5% FBS in HCEC culture medium was used as positive control in all HCEC tests. The relative viability of HCEC cultured with FD hPL exceeded 60% by MTT assay after 24 hours treatment. (Figure 1.) The HCEC migration model results demonstrated that 15% v/v FD hPL diluted into a mixture of HCEC media and Plasmalyte resulted in 96.28% migration in an in vitro wound-healing assay, while 5% diluted FD hPL yielded 69.27% migration compared to 73.48% migration in positive control cultures following 10 hours treatment. (Figure 2.) The migration in cultures exposed to the 5% and 15% diluted FD hPL were significantly better than that seen with the negative control (20.68% migration), and were comparable to the positive control of 5% FBS.

Conclusion: FD hPL contains elevated levels growth factors, EGF and PDGF-BB level compared to autologous serum or physiological eye tear film. Moreover, FD hPL has the ability to maintain HCEC viability and promote cell migration and wound healing in clinically relevant in vitro assays. FD hPL is an attractive agent for further testing in clinical trials as a topical drug product designed to promote healing of epithelial cells and corneal homeostasis in patients impacted by dry eye syndrome.

Table 1. Protein content and growth factors level in FD hPL

	Protein content (mg/ml)	EGF content (ng/mL)	PDGF-BB (ng/mL)
FD hPL	51.4±1.2	3.83±0.51	17.7±1.0
Autologous serum	0.07 [4]	0.5 [5]	5-10 [6]
Normal tear film	3.86 [7]	1.4±0.6 [8]	1.7 [9]

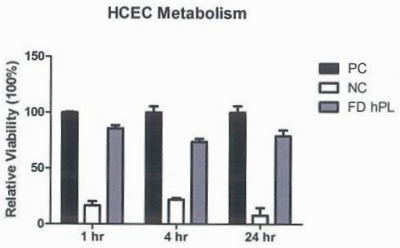

Figure 1.
FD hPL supports HCEC metabolism and cell viability. Semi-confluent HCEC cultures were established in 5% FBS in HCEC media. Cultures were washed once with PBS and media was replaced with 10% v/v FD hPL in HCEC media; 5% FBS in HCEC media with 0.3% Triton X-100 (NC) or HCEC plus 5% FBS (positive control). Viability was assessed 1, 4, and 24 hours later by the MTT assay. (*denotes $p<0.05$ representing significant differences between FD hPL groups and negative control.)

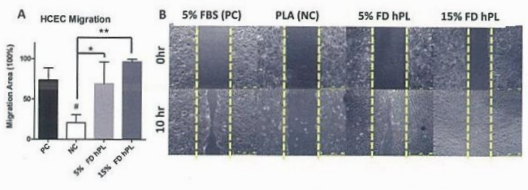

Figure 2.
FD hPL supports HCEC viability and migration in a wound healing assay
(A) Semi-confluent HCEC cultures were established in 5% FBS in HCEC media. Cultures were washed once, then

changed to 5% v/v FBS in HCEC media (PC); HCEC media with Plasmalyte (NC); 5% v/v FD hPL in Plasmalyte/HCEC media; or 15% v/v FD hPL in Plasmalyte/HCEC media. HCEC migration area was measured as the area of the original wound in the monolayer covered by growing/migrating epithelial cells. Differences in migration between the PC and NC were significant (# $p<0.05$, n=3), illustrating that this model is suitable to evaluate cell migration. Compared with the NC, both cultures with 5% FD hPL and 15% FD hPL showed significantly greater migration (* $p<0.05$, ** $p<0.01$, n=3). Moreover, both groups cultured with FD hPL groups were better or equivalent to the positive control of 5% FBS.
(B) Migration photos of HCEC wound healing model. After 10 hr treating with different condition, 15% FD hPL has almost 100% migration, better than 5% FBS, the positive control.

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